

Please amend the subject application as follows.

In the Specification:

Please replace the paragraph at page 3, lines 1-6, with the following amended paragraph:

-- Peach et al., (J. Exp. Med. (1994) 180:2049-2058) identified regions in the CTLA4 extracellular domain which are important for strong binding to CD80. Specifically, a hexapeptide motif (MYPPPY (SEQ. ID NO: 9)) in the complementarity determining region 3 (CDR3)-like region was identified as fully conserved in all CD28 and CTLA4 family members. Alanine scanning mutagenesis through the MYPPPY (SEQ ID NO: 9) motif in CTLA4 and at selected residues in CD28Ig reduced or abolished binding to CD80. --

Please replace the paragraph at page 5, lines 1-3, with the following amended paragraph:

-- Figure 6 demonstrates that L104EA29YIg is more effective than CTLA4Ig at inhibiting proliferation of ~~phytohemagglutinin~~phytohemagglutinin (PHA) stimulated monkey T cells as described in Example 2, *infra*. --

Please replace the paragraph at page 5, lines 21-24, with the following amended paragraph:

-- Figures 11A and 11B illustrate a ribbon diagram of the CTLA4 extracellular Ig V-like fold generated from the solution structure determined by NMR spectroscopy. FIG. 11B shows an expanded view of the S25-R33 region and the MYPPPY (SEQ ID NO: 9) region indicating the location and side-chain orientation of the avidity enhancing mutations, L104 and A29. --

Please replace the paragraph at page 6, lines 4-22, with the following amended paragraph:

-- As used herein "wild type CTLA4" has the amino acid sequence of naturally occurring, full length CTLA4 (U.S. Patent Nos. 5,434,131, 5,844,095, 5,851,795), or the extracellular domain ~~thereof~~thereof, which binds CD80 and/or CD86, and/or interferes with CD80 and/or CD86 from binding their ligands. In particular embodiments, the extracellular domain of wild type CTLA4 begins with methionine at position +1 and ends at aspartic acid at position +124, or the extracellular domain of wild type CTLA4 begins with alanine at position -1 and ends at aspartic acid at position +124. Wild type CTLA4 is a cell surface protein, having an N-terminal extracellular domain, a transmembrane domain, and a C-terminal cytoplasmic domain. The extracellular domain binds to target antigens, such as CD80 and CD86. In a cell, the naturally occurring, wild type CTLA4 protein is translated as an immature polypeptide, which includes a signal peptide at the N-terminal end. The immature polypeptide undergoes post-translational processing, which includes cleavage and removal of the signal peptide to generate a CTLA4 cleavage product having a newly generated N-terminal end that differs from the N-terminal end in the immature form. One skilled in the art will appreciate that additional post-translational processing may occur, which removes one or more of the amino acids from the newly generated N-terminal end of the CTLA4 cleavage product. The mature form of the CTLA4 molecule includes the extracellular domain of CTLA4, or any portion thereof, which binds to CD80 and/or CD86. --

Please replace the paragraph at page 19, lines 12-30, and continuing on page 20, lines 1-5, with the following amended paragraph:

-- The present invention further provides methods for treating immune system diseases and tolerance induction. In particular embodiments, the immune system diseases are mediated by CD28- and/or CTLA4-positive cell interactions with CD80/CD86-positive cells. In a further embodiment, T cell interactions are inhibited. Immune system diseases include, but are not limited to, autoimmune diseases, ~~immunoproliferative diseases,~~

immunoproliferative diseases, and graft-related disorders. These methods comprise administering to a subject the soluble CTLA4 mutant molecules of the invention to regulate T cell interactions with the CD80- and/or CD86-positive cells. Alternatively, a CTLA4 mutant hybrid having a membrane glycoprotein joined to a CTLA4 mutant molecule can be administered. Examples of graft-related diseases include graft versus host disease (GVHD) (e.g., such as may result from bone marrow transplantation, or in the induction of tolerance), immune disorders associated with graft transplantation rejection, chronic rejection, and tissue or cell allo- or xenografts, including solid organs, skin, islets, muscles, hepatocytes, neurons. Examples of immunoproliferative diseases include, but are not limited to, psoriasis; T cell lymphoma; T cell acute lymphoblastic leukemia; testicular angiocentric T cell lymphoma; benign lymphocytic angiitis; and autoimmune diseases such as lupus (e.g., lupus erythematosus, lupus nephritis), Hashimoto's thyroiditis, primary myxedema, Graves' disease, pernicious anemia, autoimmune atrophic gastritis, Addison's disease, diabetes (e.g. insulin dependent diabetes mellitis, type I diabetes mellitis), good pasture's syndrome, myasthenia gravis, pemphigus, Crohn's disease, sympathetic ophthalmia, autoimmune uveitis, multiple sclerosis, autoimmune hemolytic anemia, idiopathic thrombocytopenia, primary biliary cirrhosis, chronic action hepatitis, ulceratis colitis, Sjogren's syndrome, rheumatic diseases (e.g., rheumatoid arthritis), polymyositis, scleroderma, and mixed connective tissue disease. --

Please replace the paragraph at page 20, lines 6-16, with the following amended paragraph:

-- The present invention further provides a method for inhibiting solid organ and/or tissue transplant rejections by a subject, the subject being a ~~recipient~~ recipient of transplant tissue. Typically, in tissue transplants, rejection of the graft is initiated through its recognition as foreign by T cells, followed by an immune response that destroys the graft. The soluble CTLA4 mutant molecules of this invention, by inhibiting T lymphocyte proliferation and/or cytokine secretion, may result in reduced tissue destruction and

induction of antigen-specific T cell unresponsiveness may result in long-term graft acceptance without the need for generalized immunosuppression. Furthermore, the soluble CTLA4 mutant molecules of the invention can be administered with other pharmaceuticals including, but not limited to, corticosteroids, cyclosporine, rapamycin, mycophenolate mofetil, azathioprine, tacrolimus, basiliximab, and/or other biologics. --

Please replace the paragraph at page 37, lines 16-29, and continuing at page 38, lines 1-7, with the following amended paragraph:

-- The solution structure of the extracellular IgV-like domain of CTLA4 has recently been determined by NMR spectroscopy (Metzler et al., (1997) Nature Struct. Biol., 4:527-531. This allowed accurate location of leucine 104 and alanine 29 in the three dimensional fold (FIG. 11A-B). Leucine 104 is situated near the highly conserved MYPPPY (SEQ ID NO: 9) amino acid sequence. Alanine 29 is situated near the C-terminal end of the S25-R33 region, which is spatially adjacent to the MYPPPY (SEQ ID NO: 9) region. While there is significant interaction between residues at the base of these two regions, there is apparently no direct interaction between L104 and A29 although they both comprise part of a contiguous hydrophobic core in the protein. The structural consequences of the two avidity enhancing mutants were assessed by modeling. The A29Y mutation can be easily accommodated in the cleft between the S25-R33 region and the MYPPPY (SEQ ID NO: 9) region, and may serve to stabilize the conformation of the MYPPPY (SEQ ID NO: 9) region. In wild type CTLA4, L104 forms extensive hydrophobic interactions with L96 and V94 near the MYPPPY (SEQ ID NO: 9) region. It is highly unlikely that the glutamic acid mutation adopts a conformation similar to that of L104 for two reasons. First, there is insufficient space to accommodate the longer glutamic acid side chain in the structure without significant perturbation to the S25-R33 region. Second, the energetic costs of burying the negative charge of the glutamic acid side chain in the hydrophobic region would be large. Instead, modeling studies predict that the glutamic acid side chain flips out on to the surface where its charge can be

stabilized by solvation. Such a conformational change can easily be accommodated by G105, with minimal distortion to other residues in the regions. --

Please replace the paragraph at page 40, lines 6-11, with the following amended paragraph:

-- The effects of L104EA29YIg and CTLA4Ig on monkey mixed lymphocyte response (MLR) are shown in Figure 6. Peripheral blood mononuclear cells (PBMC'S; 3.5×10^4 cells/well from each monkey) from 2 monkeys were purified over lymphocyte separation medium (LSM) and mixed with 2 μ g/ml ~~phytohemagglutinin~~phytohemagglutinin (PHA). The cells were stimulated 3 days then pulsed with radiolabel 16 hours before harvesting. L104EA29YIg inhibited monkey T cell proliferation better than CTLA4Ig. --